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Updates from MHDL

New Bordetella Multiplex Real-time PCR Assay at Milwaukee Health Department Laboratory

The diagnosis of pertussis ("whooping cough") at an early stage of illness is important in order to institute therapy and prevent transmission. Although most commonly caused by *Bordetella pertussis*, pertussis-like respiratory illness can also be caused by other *Bordetella* species including *B. parapertussis*, *B. bronchiseptica* and *B. holmesii*. In the past, the MHD Laboratory used real-time PCR for nucleic acid amplification of *IS481* region to detect *Bordetella pertussis* and *IS1001* region to detect *B. parapertussis* in nasopharyngeal swabs collected from symptomatic patients. Although the assay was more sensitive than culture and used for rapid detection of *B. pertussis*, it had the limitation for the possible detection of *B. holmesii* due to very high sequence similarity of the *IS481* target gene. Recently, we have validated a multiplex real-time PCR assay for the detection and differentiation of *B. pertussis, B. parapertussis* and *B. holmesii* from clinical specimens. The assay was developed at CDC and targets the *hIS1001* insertion sequence for *B. holmesii* and the S1 subunit of the toxin gene (*ptxS1)* of *B. pertussis.* The assay is highly sensitive, specific and was evaluated on multiple *Bordetella*, non-*Bordetella* species, as well as several common respiratory bacterial pathogens for any cross-reaction. The assay can be used with culture, other diagnostic assays or to determine infection due to *B. pertussis, B. parapertussis* and *B. holmesii* in clinical specimens from patients with symptoms of pertussis.

Laboratory Confirmed Influenza (by Week) at the City of Milwaukee Public Health Laboratory

Laboratory Confirmed Influenza by Week: City of Milwaukee Public Health Laboratory					
October 1, 2010 to April 16, 2011			Positives by Type		
Week Ending	**No. Tested**	**No. Pos.**	**2009 A/H1N1**	**A/H3**	**B**
10/01/10 to 01/01/11	59	0	0	0	0
01/08/11	0	0	0	0	0
01/15/11	7	2	2	0	0
01/22/11	4	1	1	0	0
01/29/11	10	5	2	2	1
02/05/11	32	28	22	3	3
02/12/11	42	16	13	3	0
02/19/11	17	6	5	0	1
02/26/11	18	9	8	0	1
03/05/11	21	8	8	0	0
03/12/11	12	8	7	0	1
03/19/11	13	5	3	1	1
03/26/11	4	0	0	0	0
04/02/11	8	0	0	0	0
04/09/11	5	1	0	0	1
04/16/11	8	0	0	0	0
Total	**260**	**89**	**71**	**9**	**9**

SUMMARY OF CONFIRMED INFECTIONS

Clinical and Environmental Microbiology

The April 2011 issue presents the laboratory diagnosis of some of the infectious diseases and the reference microbiology work done in this laboratory during March 2011 and new cases of syphilis in City of Milwaukee during March 2011.

Syphilis

Test	Total	Test	Total
RPR Reactive	0	TPPA Reactive	6
VDRL Reactive	13	Darkfield Positive	0

New Cases of Syphilis

Stage	Number of Cases	
	March 2011	March 2010
Primary syphilis	0	0
Secondary syphilis	0	2
Early latent	2	4
Late latent	0	5
Total	2	11

Source: Wisconsin Division of Health

Gonorrhea Antimicrobial Susceptibility Testing

Number Tested	Decreased Susceptible (DS) / Resistant (R) Antibiotics			
	Ciprofloxacin	Cefixime	Ceftriaxone	Azithromycin
32	2	0	0	0

Isolates Other Than *N. gonorrhoeae*

Organism	Site	Number Isolates	Organism	Site	Number Isolates
Ureaplasma urealyticum	Genital	6	*Mycoplasma hominis*	Genital	2

Enteric Parasites Identified

Age	Sex	Parasite
55	F	*Ascaris lumbricoides*
		Giardia lamblia
17	F	*Blastocystis hominis*

23	F	*Blastocystis hominis*
18	F	*Blastocystis hominis*
13	F	*Blastocystis hominis*
35	F	*Blastocystis hominis*
		Entamoeba histolytica/Entamoeba dispar
24	M	*Blastocystis hominis*
		Endolimax nana
44	F	*Blastocystis hominis*
		Entamoeba hartmanni
25	M	*Endolimax nana*
24	M	*Entamoeba coli*
23	F	*Entamoeba coli*
20	F	*Entamoeba coli*
21	M	*Entamoeba hartmanni*
19	F	*Endolimax nana*
		Iodamoeba buetschlii
55	F	*Giardia lamblia*
16	M	*Giardia lamblia*
56	F	*Giardia lamblia*

Mycobacterial Infections

Age	Sex	Test Results				Identification
		Sputum Smear	Culture	DNA Probe	PCR	
61	F	-	+	ND	-	*M. fortuitum* group
55	M	-	+	ND	-	*M. mucogenicum*

Reference Cultures

Age	Sex	Source	Identification
24	M	Stool	*E. coli* other than *E. coli* O157:H7
23	M	Throat	*Neisseria meningitides*
34	M	Urethra	*Neisseria meningitides*
21	F	Vagina	*Neisseria gonorrhoea*
24	M	Stool	*Salmonella* Newport
64	F	Urine	*Salmonella* Newport
25	F	Stool	*Salmonella* Enteritidis
67	M	Stool	*Salmonella* Enteritidis
41	F	Ankle, left	*Serratia marcescens*
27	M	Stool	*Shigella flexneri* type 2

Virology and Molecular Science

Virus Isolations from Clinical Specimens

Age	Sex	Source	Symptoms	Agent
20	F	NP	Influenza-like illness	2009 influenza A H1N1
20	F	Throat	Fever, headache, sore throat	2009 influenza A H1N1
19	M	Throat and NP	Fever (102.8°), cough, sore throat, headache, congestion, myalgia	2009 influenza A H1N1
19	M	Swab	Fever, headache, cough, sore throat	2009 influenza A H1N1
25	F	Throat and NP	Fever, cough	2009 influenza A H1N1
19	M	Throat and NP	Fever, myalgia, cough, sore throat	2009 influenza A H1N1
22	M	Throat and NP	Fever, headache, sore throat	2009 influenza A H1N1
46	F	Throat and NP	N/A	2009 influenza A H1N1
23	M	Throat and NP	Fever, diarrhea, nausea, cough, myalgia	2009 influenza A H1N1
20	F	Throat and NP	Fever (100.9°), cough, sore throat, myalgia	2009 influenza A H1N1
26	F	Throat and NP	Cough, headache, myalgia	2009 influenza A H1N1
20	M	Throat and NP	Fever (101°), headache, nausea, myalgia, cough, sore throat	2009 influenza A H1N1
21	M	Throat and NP	R/O influenza	2009 influenza A H1N1
21	F	Throat and NP	Fever (103°), cough, sore throat, headache	2009 influenza A H1N1
18	F	Throat and NP	Fever, headache, myalgia, cough	2009 influenza A H1N1
19	M	Throat and NP	Fever (102.6°), myalgia, cough, nausea/vomiting, headache	2009 influenza A H1N1
20	M	Throat and NP	Cough, sore throat	2009 influenza A H1N1
20	F	Throat and NP	Fever, myalgia, cough, sore throat	2009 influenza A H1N1
29	M	Throat and NP	Fever (101.4°), cough, myalgia, sore throat, vomiting, headache, SOB	2009 influenza A H1N1
26	F	Throat and NP	Fever, cough, myalgia, sore throat, headache, URI	2009 influenza A H1N1
22	M	Throat and NP	ARD, sore throat, headache	2009 influenza A H1N1
20	M	Throat and NP	R/O influenza	2009 influenza A H1N1
25	F	Throat	Fever, vomiting, diarrhea, cough, sore throat	2009 influenza A H1N1
22	F	NP	Fever (102.1°), sore throat	Influenza A (H3)
20	F	Throat and NP	Fever, headache, cough	Influenza B
22	M	Throat and NP	Cough, sore throat	Influenza B
20	F	Throat and NP	Not Provided	Influenza B
22	M	Throat	Fever (101°) Documented H1N1 in early February, 2011	Influenza B
21	F	Throat and NP	Fever (101.6°), cough, sore throat, headache	Adenovirus
41	M	Sputum	Fever, URI	Human metapneumovirus
22	M	Throat	Fever, headache, cough	Parainfluenza virus type-1
19	F	Throat and NP	R/O influenza	Parainfluenza virus type-3
19	F	Swab	Fever, ARD, sore throat	Parainfluenza virus type-3
25	F	Throat and NP	ARD	Parainfluenza virus type-3
86	F	Swab	Hoarseness, cough, crackles, dyspnea, pneumonia	Parainfluenza virus type-3
20	M	Throat and NP	Fever, cough, myalgia, sore throat	Respiratory Syncytial Virus

21	M	Throat and NP	Fever, nausea, cough, myalgia	Rhinovirus
21	M	Throat and NP	Acute pharyngitis (negative strep screen), sore throat, headache	Rhinovirus

Influenza Real-time RT-PCR Testing

Samples Tested	2009 Influenza A (H1N1) Positive	Influenza A (H3) Positive	Influenza B Positive
51	20	1	2

Herpes Simplex Virus Isolations

Agent	Number of Isolates
Herpes Simplex type 1	12
Herpes Simplex type 2	11

Molecular Amplification and PCR

Agent	Method	Tested	Positive	% Positive
Norovirus	RT-PCR	1	0	0%
Bordetella pertussis/parapertussis	RT-PCR	1	0	0%
Toxigenic *Clostridium difficile*	RT-PCR	2	1	50%
Chlamydia trachomatis	Gen-Probe Aptima	627	89	15.6%
Neisseria gonorrhoeae	Gen-Probe Aptima	847	67	7.9%

Respiratory Virus Surveillance:

Respiratory Virus Panel Test Results		
Virus	**Positives**	**Percent**
Human Rhinovirus (HRV)	**15**	**6.8%**
Coronavirus OC43	**7**	**3.2%**
Parainfluenza virus 3 (PIV3)	**7**	**3.2%**
Adenovirus Type E (Adeno E)	**4**	**1.8%**
Adenovirus Type E (Adeno E)	**4**	**1.8%**
Respiratory Syncytial virus (RSV)	**4**	**1.8%**
Coronavirus NL63	**3**	**1.4%**
Adenovirus Type C (Adeno C)	**2**	**0.9%**
Human metapneumovirus (hMPV)	**2**	**0.9%**
Parainfluenza virus 1 (PIV1)	**1**	**0.5%**

Samples tested: 220 (Sept. 1, 2010 – April 12, 2011)

DNA Sequencing: The MHD laboratory uses 16S rRNA and the D2 region of the 26S rRNA genes for DNA sequence-based microbial identification of selective reference bacteria and fungal isolates.

Reference Microbe	Age	Sex	Source	Target gene	Final Identification
Bacteria	2m	F	Wound	16S rRNA	*Clostridium tertium*
Bacteria	31	M	Blood	16S rRNA	*Paenibacillus* species
Bacteria	41	F	Ankle, L	16S rRNA	*Serratia marcescens*